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6.1 Gene Expression in Midgut tissues of *Diaphorina citri*: *Application to biology and vector control*

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Digestive enzymes advantageous for feeding from plants include the specific digestive enzymes amylase and pectinase (Cohen 1996). Plant feeding Hemiptera, like the Asian citrus psyllid, AsCP, *Diaphorina citri,* Kuwayama (Hemiptera: Psyllidae) are specialized feeders in that they feed primarily from plant phloem. To gain an understanding of *D. citri* feeding and digestion we elected to examine the genes expressed in the midgut tissues. During feeding *D. citri* can transmit bacteria, specifically *Candidatus* Liberibacter asiaticus which is considered the primary cause of the citrus disease Huanglongbing, HLB. Psyllid expressed sequence tags, ESTs, provide useful information on the midgut genes being expressed within these specific tissues. This information enables the identification of many of the genes and proteins having key roles in psyllid digestion. Thus we undertook a 5′ end sequencing project from adult *D. citri*. Through these and other efforts ~17,000 ESTs have been produced from *D. citri* (Hunter et. al. 2005-2008). The Midgut cDNA library is providing valuable information from which researchers are now developing new management strategies based on emerging RNAi methodologies to disrupt psyllid feeding.

Materials and Methods

Asian citrus psyllids, *D. citri,* were obtained from a colony established from field caught adults, maintained at the USDA, ARS, U.S. Horticultural Research Laboratory, Ft. Pierce, FL. Insects were reared on *Murraya paniculata* (L.) 'Orange-jasmine' seedlings in screen cages contained in an insectary, and held at 25°C, 16 L: 8 D. Three hundred psyllid midguts were dissected out in RNAlater, then homogenized and total RNA extracted. cDNA was synthesized using Stratagene ZAP-cDNA Synthesis Kit (Stratagene, La Jolla, CA, USA). Mass excision of the amplified library was carried out using Ex-Assist helper phage (Stratagene, La Jolla, CA, USA) and bacterial clones containing excised pBluescript SK(+) phagemids were recovered by random colony selection. Sequencing performed at the USDA, ARS, U.S. Hort. Res. Lab, genomic lab, Ft. Pierce, FL. Reactions were performed using the ABI PRISM® BigDye™ Primer Cycle Sequencing Kit (Applied Biosystems). Reactions were prepared in 96-well format using the Biomek2000™ liquid handling robot (Beckman Coulter, Inc., USA). Sequencing reaction products were precipitated with 70% isopropanol, resuspended in 15 µL sterile water and loaded onto an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Of the 7,800 ESTs, 6,200 were validated for submission after base calling, quality trimming, vector trimming and sequence fragment alignments were performed by Sequencher™ (Gene Codes Corp., USA). Low-quality bases (quality score <12) were trimmed from both ends of sequences. Assembly parameters were set using a minimum overlap of 30 bp, match spacing of 150, minimum length of 150bp and 90% identity. Putative sequence identity was determined based on BLAST similarity searches (BLASTX and BLASTn) using NCBI, Batch Blast (June 2008). Available at GenBank, dbEST, Accession numbers: FK254041- FK260232. GenBank http://www.ncbi.nlm.nih.gov

Results and Discussion

Most insects produce a range of digestive proteases, which may be regulated in direct response to food or produced constitutively throughout the life of the insect (Terra & Ferreira, 1994; Lehane *et al.* 1996). Our results identified many different enzymes within *D. citri*, (Fig 1) Biological Process: Distribution of *Diaphorina citri* transcripts from Blastx analysis using a summary with a minimum of 70 sequences per category resulted in these categories in descending order: Response to stress, 227; Proton transport 167; Response to chemical stimuli 157; Glycolysis 139; Instar/pupal development 137; Larval development 129; Mesoderm development 124; Intracellular signaling cascade 118; Proteolysis 117; Oogenisis 113; Behavior 111; Amino acid metabolic process 111;Proteins amino acid phosphorylation 109; Negative regulation of cellular process 109; Cytokinesis 107; DNA metabolic process 105; and Monocaboxylic acid metabolism 104. While regulation of enzyme production and release appears to be influenced by feeding, it is likely also to involve a combination of hormonal release, paracrine activity and direct feeding mechanisms. This has been observed in the beetle, *C. zealandica*, where addition of serine protease inhibitors to the diet caused trypsin and leucine aminopeptidase activities to increase (Dymock *et al.*, 1992). Research has also shown that protease activity can be affected by disease infection of *C. zealandica* larvae by the bacteria *Serratia* spp. containing a specific plasmid results in a rapid elimination of serine protease activity in the midgut (Grkovic *et al.* 1995; Hurst e*t al.*, 2000).

Disruption of insect-specific physiological processes has been identified as a useful route for the development of novel management strategies against insect pests. One such area is disruption is to affect the specific enzymes used in digestion which can be inhibited or blocked at synthesis by RNAi approaches. This tactic is promising but has proven more difficult than first envisaged as insects generally produce a range of enzymes under control of multiple genes, thus in-depth genetic studies need to be completed to identify specific enzymes and their biological pathways. The availability of these sequences now enables investigations into these important questions regarding *D. citri* digestion and biology. The *D. citri* gene expression data set advances what is currently known about psyllid digestion. The enzymes identified further provide possible genetic targets to be used to alter *D. citri* digestive enzymes and physiological processes.

In summary, a gene expression library was made from the alimentary tract of adult Asian citrus psyllids, AsCP. Analysis of the expressed sequence tags produced a gene dataset of 7,800 EST's. Enzymes important to digestion and feeding on a phloem diet were identified including several serine proteases, hydrolases, and cathepsins. These and other transcripts with significant homology (E-value $\leq 10\text{-}^{20}$ or better) were identified through homology searches to other known insect genomes. Use of genomics approaches has enabled us to identify some of the genetic basis of psyllid digestion and pathogen interactions. Further genomic analyses of the AsCP, *Diaphorina citri*, will advance our understanding of the psyllid/phloem/bacterium interactions which may be linked to the acquisition and transmission of the pathogenic bacterium Liberibacter asiaticus, associated with the citrus disease Huanglongbing (HLB). However, a much greater understanding of psyllid genomics is still needed. Continued development of these genetic products will set the foundation for further functional genomic studies to isolate AsCP specific genes to be targeted to reduce the spread of HLB, citrus greening disease, and to reduce psyllid populations using environmentally friendly, highly specific management strategies.

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Fig. 1 Biological Process: Distribution of *Diaphorina citri* transcripts blastx summary for minimum of 70 sequences per category. Highest Categories in descending order: Response to stress, 227; Proton transport 167; Response to chemical stimuli 157; Glycolysis 139; Instar/pupal development 137; Larval development 129; Mesoderm development 124; Intracellular signaling cascade 118; Proteolysis 117; Oogenisis 113; Behavior 111; Amino acid metabolic process 111;Proteins amino acid phosphorylation 109; Negative regulation of cellular process 109; Cytokinesis 107; DNA metabolic process 105; Monocaboxylic acid metabolism 104. (Blast2Go).